Multiple Sequence Alignments: Purpose, Assessment and Application

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Multiple Sequence Alignment (MSA)

Definition:

A multiple sequence alignment (**MSA**) is a sequence alignment of three or more biological sequences, generally protein, DNA, or RNA.

Importance:

Homology across genome sequences can be inferred from the MSA and used to assess gene or genome evolution, diversity, structure and function.

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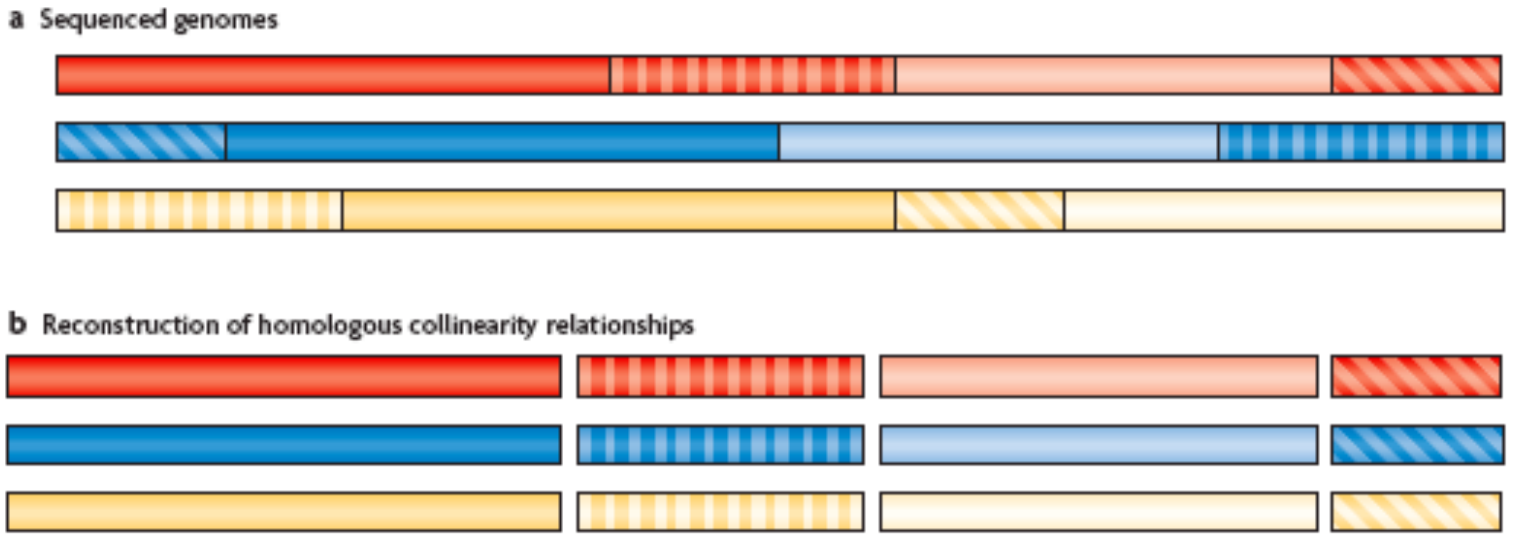
Alignment Criteria: Optimal collinearity of homologous regions



MARGULIES AND BIRNEY NAT REV 2008

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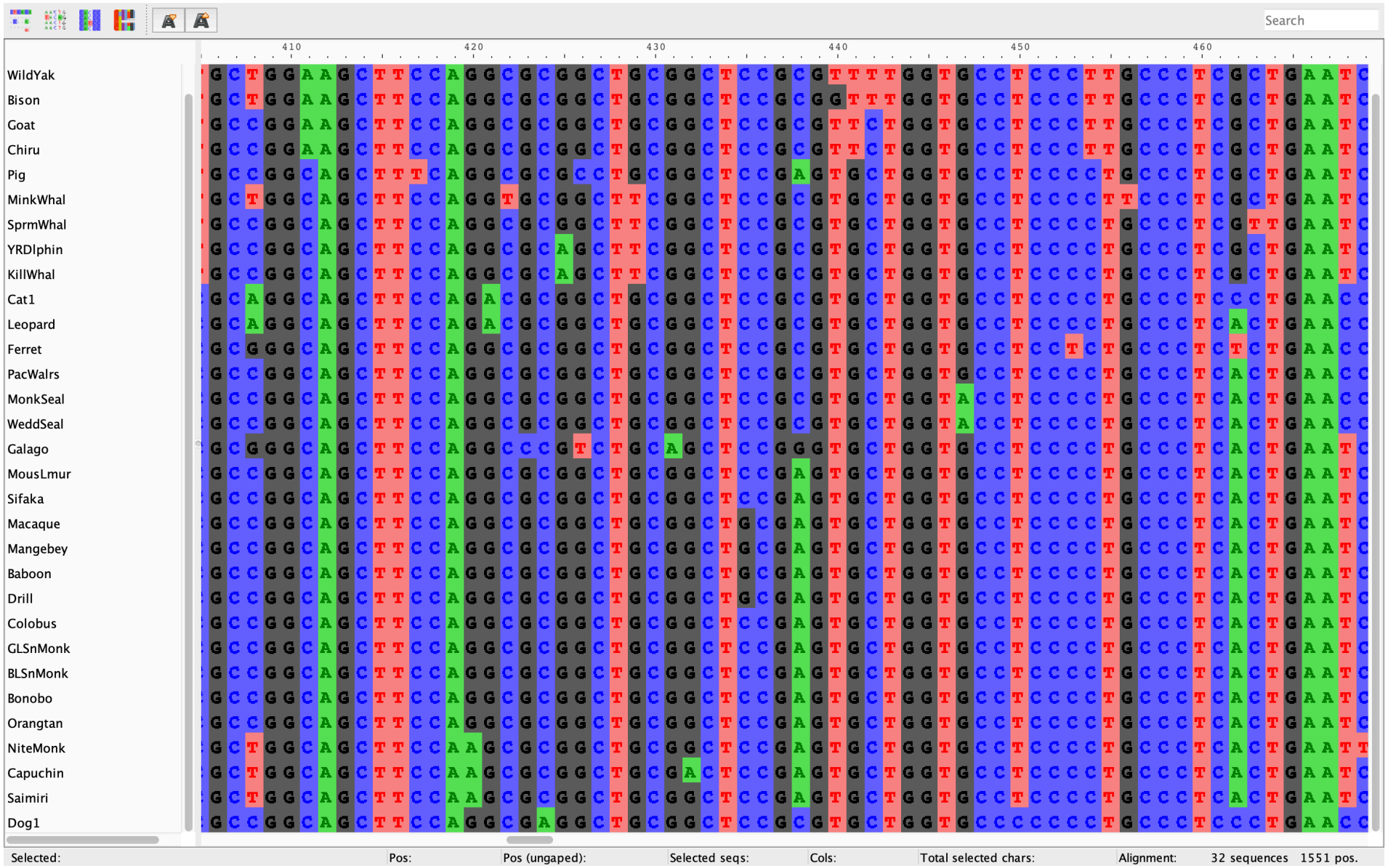
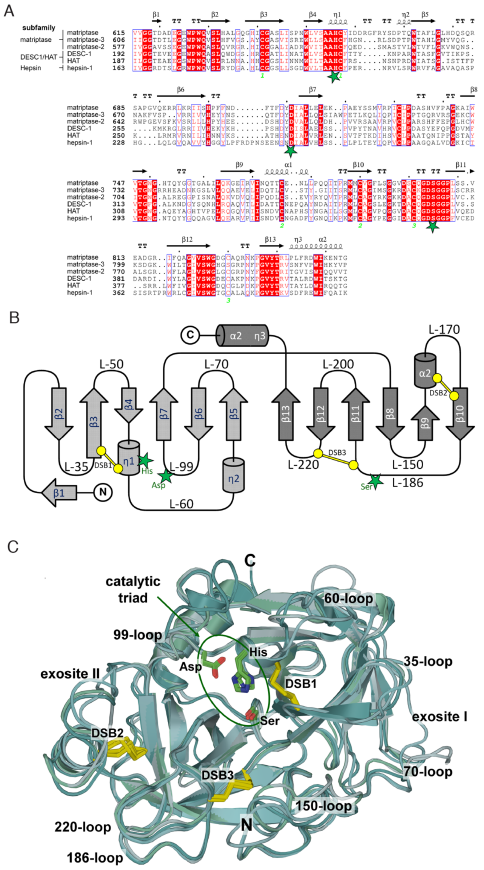

Search
410
420
430
440
450
460
WildYak
Bison
Goat
Chiru
Pig
MinkWhal
SprmWhal
YRDlphin
KillWhal
Cat1
Leopard
Ferret
PacWalrs
MonkSeal
WeddSeal
Galago
MousLmur
Sifaka
Macaque
Mangebey
Baboon
Drill
Colobus
GLSnMonk
BLSnMonk
Bonobo
Orangtan
NiteMonk
Capuchin
Saimiri
Dog1
Selected:
Pos:
Pos (ungaped):
Selected seqs:
Cols:
Total selected chars:
Alignment:
32 sequences 1551 pos.

Crucial Input for Comparative Genomic Applications

Comparing within Genomes

Gene families

Structure, function, evolution



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Crucial Input for Comparative Genomic Applications

Host:

Individuals

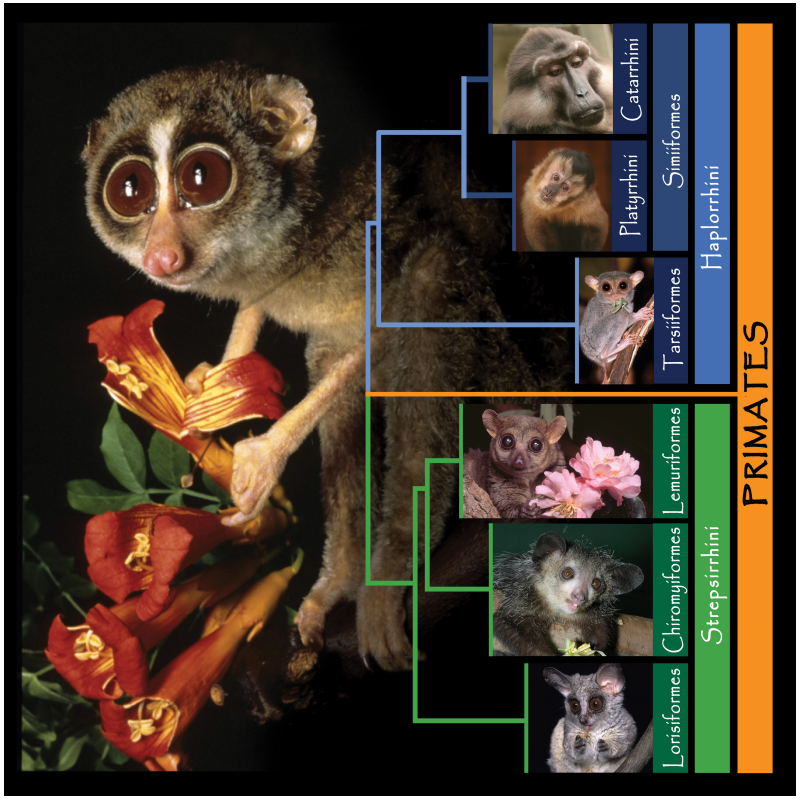
Populations

Species

Pathogen:

Within host

Between host



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True simultaneous alignment would be multi-dimensional and prohibitively computer intensive

General components to alignment process of most programs:

Series of pairwise alignments combined into multiple alignments.

Guide trees constructed for intermediate levels of addition and tested.

Different alignment programs use different ways of creating the final multiple sequence file using both global and local methods

Traditional approach uses a progressive alignment based on evolutionary relationships of sequences

Iterative alignment methods (MCMC) that also include biological features to help align (e.g. circular genomes)

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Commonly Used MSA Programs

- MUSCLE MUltiple Sequence Comparison by Log- Expectation
- ClustalX
- Clustal Omega*
- MAFFT Multiple Alignment Using Fast Fourier Transform*
- PRANK: Probabilistic Alignment Kit*

Each offers a different criteria or algorithm for the intermediate stages in refinement (guide trees and partial alignments) between input and final form.

Because of this, some may work better than others for your data set.

Speed versus accuracy

*Scalable for large datasets such as whole genomes

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Translation Alignments with Codons

- Codon Triplet: 1st, 2nd, 3rd Positions

  Different Probabilities for Synonymous (dS) and Nonsynonymous (dN) Substitutions

  Non-degenerate (2nd position): all encode nonsynonymous (amino acid altering) substitutions

  Two-fold degenerate (1st position): both nonsynonymous and synonymous substitutions

  Four-fold (3rd position): all synonymous

- Translation into amino acid residues to aid alignment

  Applications:

  Difficult alignment from genomes characterized by:

  Ancient divergence events

  Rapidly mutating genomes

  Or

  Identification of conserved motifs for coding regions

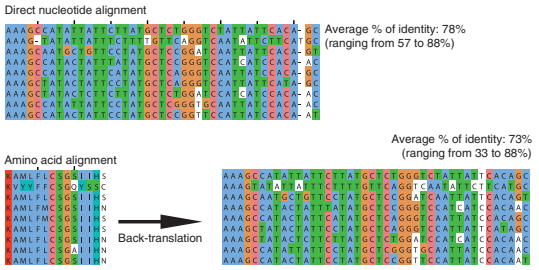

Figure 1. Example illustrating the different performance of the direct and back-translated nucleotide alignments (multiple alignments were built with Muscle with default parameters).

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Evolution of Amino Acids

Underlying assumption:

Construct alignment that minimize cost preserve physical and chemical properties of sequence

Types of probability matrices:

1) Empirical Data Models
PAM-DayHoff, JTT, WAG

2) Organelle specific matrices:
MtREV
cpREV10, cpREV64

3) Pathogens
FLU Influenza

		Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
		A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
Ala	A	13	6	9	9	5	8	9	12	6	8	6	7	7	4	11	11	11	2	4	9
Arg	R	3	17	4	3	2	5	3	2	6	3	2	9	4	1	4	4	3	7	2	2
Asn	N	4	4	6	7	2	5	6	4	6	3	2	5	3	2	4	5	4	2	3	3
Asp	D	5	4	8	11	1	7	10	5	6	3	2	5	3	1	4	5	5	1	2	3
Cys	C	2	1	1	1	52	1	1	2	2	2	1	1	1	1	2	3	2	1	4	2
Gln	Q	3	5	5	6	1	10	7	3	7	2	3	5	3	1	4	3	3	1	2	3
Glu	E	5	4	7	11	1	9	12	5	6	3	2	5	3	1	4	5	5	1	2	3
Gly	G	12	5	10	10	4	7	9	27	5	5	4	6	5	3	8	11	9	2	3	7
His	H	2	5	5	4	2	7	4	2	15	2	2	3	2	2	3	3	2	2	3	2
Ile	I	3	2	2	2	2	2	2	2	2	10	6	2	6	5	2	3	4	1	3	9
Leu	L	6	4	4	3	2	6	4	3	5	15	34	4	20	13	5	4	6	6	7	13
Lys	K	6	18	10	8	2	10	8	5	8	5	4	24	9	2	6	8	8	4	3	5
Met	M	1	1	1	1	0	1	1	1	1	2	3	2	6	2	1	1	1	1	1	2
Phe	F	2	1	2	1	1	1	1	1	3	5	6	1	4	32	1	2	2	4	20	3
Pro	P	7	5	5	4	3	5	4	5	5	3	3	4	3	2	20	6	5	1	2	4
Ser	S	9	6	8	7	7	6	7	9	6	5	4	7	5	3	9	10	9	4	4	6
Thr	T	8	5	6	6	4	5	5	6	4	6	4	6	5	3	6	8	11	2	33	6
Trp	W	0	2	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	55	1	0
Tyr	Y	1	1	2	1	3	1	1	1	3	2	2	1	2	15	1	2	2	3	31	2
Val	V	7	4	4	4	4	4	4	4	5	4	15	10	4	10	5	5	5	72	4	17

A PAM250 matrix. Each column has been adjusted so that the columns sum to 100.

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How do we know it worked?

?

Why would it fail?

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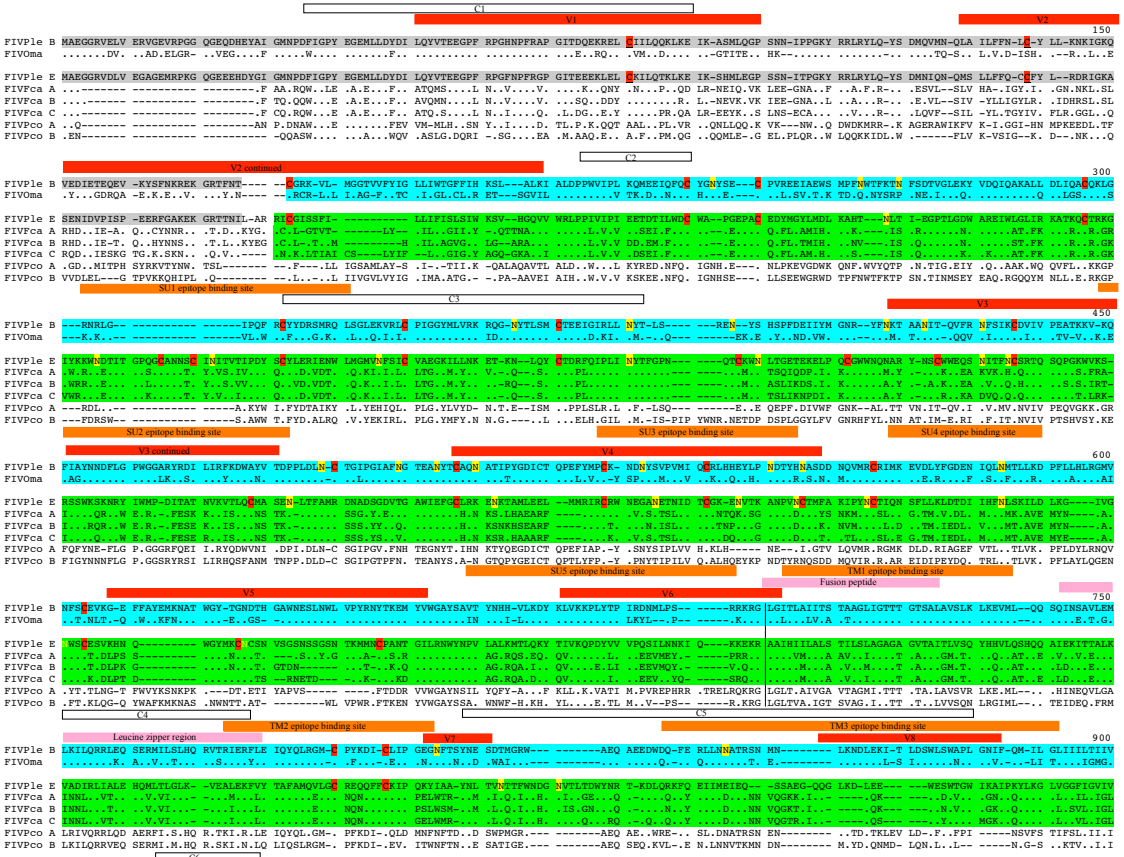
Recombination in Alignments



BMC Genomics

BioMed Central

Research article

Open Access

Genomic organization, sequence divergence, and recombination of feline immunodeficiency virus from lions in the wild

Jill Pecon-Slattery*[1], Carrie L McCracken[2], Jennifer L Troyer[2], Sue VandeWoude[3], Melody Roelke[2], Kerry Sondgeroth[4], Christiaan Winterbach[5], Hanlie Winterbach[5] and Stephen J O'Brien[4]

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Subtype B Serengeti Population: Wild type

Subtype E Okavango Delta Population: Recombinant

leader and
ORF rev exon 1

env surface and
transmembrane

putative RRE

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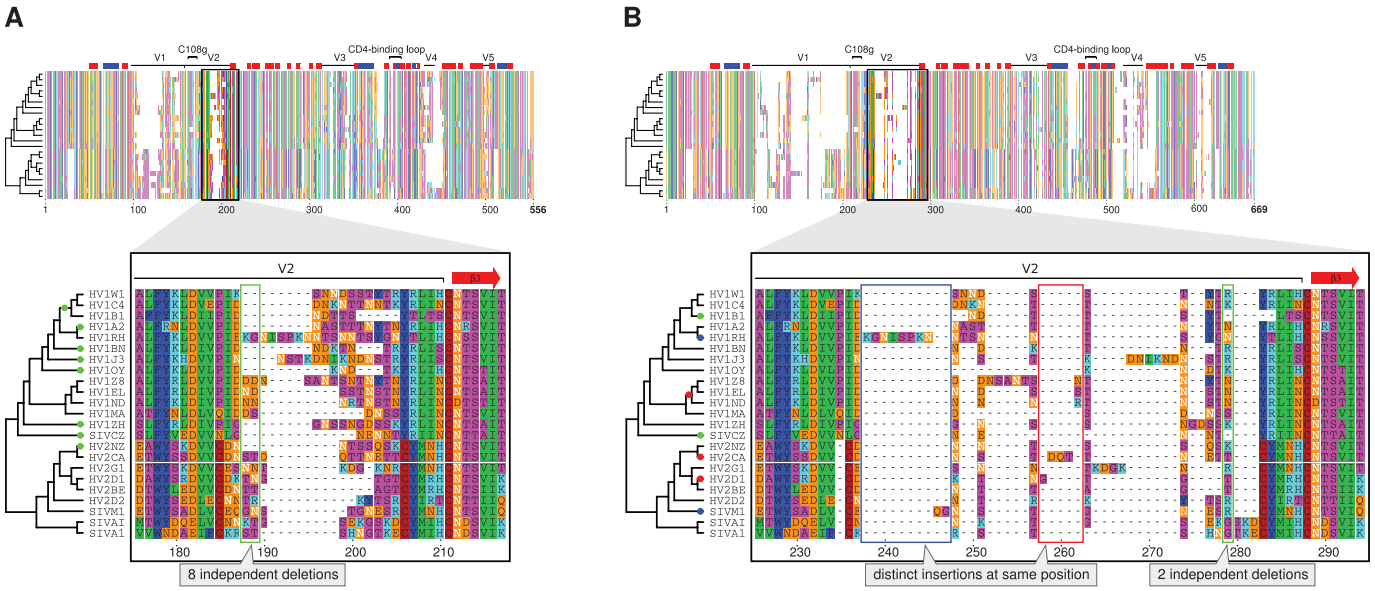
Insertion/Deletions-Can we align and interpret correctly?

SIV/HIV alignment gp120: Hypervariable regions with excessive rates of insertion/deletion

Clustal Omega

PRANK+F



Phylogeny-Aware Gap Placement Prevents Errors in Sequence Alignment and Evolutionary Analysis Ari Löytynoja, et al. Science 320, 1632 (2008)

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Larger-Sized Indels: (>150-200 bp) Can Confound Alignment Process

RepeatMasker database if needed

Currently over 56% of human genomic sequence is identified and masked by the program.

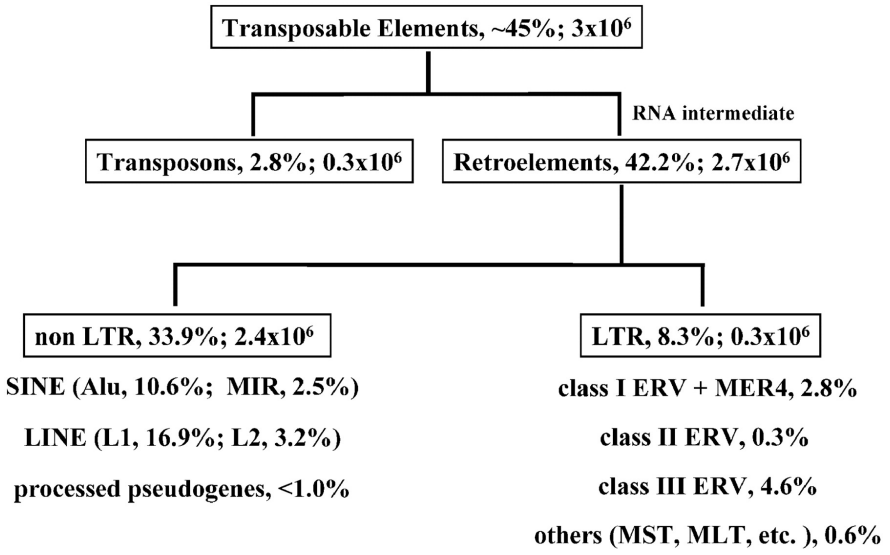
Based on curated libraries of repeats RepBase, dfam.

https://www.girinst.org

This may not work for new genome sequences from novel organisms

May wish to understand the mechanisms or functional outcome of TE insertion events

Transposable Elements Human Genome



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How it should look if alignment method recognized large indels

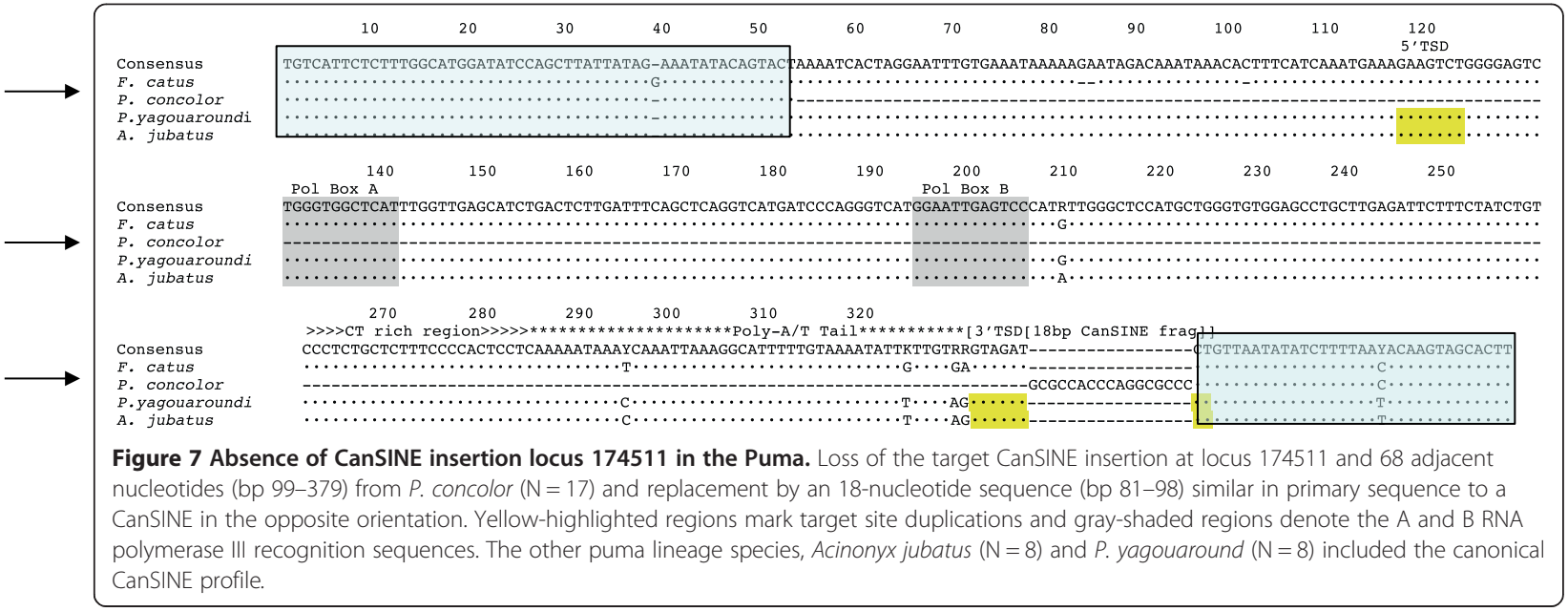

Figure 7 Absence of CanSINE insertion locus 174511 in the Puma. Loss of the target CanSINE insertion at locus 174511 and 68 adjacent nucleotides (bp 99–379) from *P. concolor* (N = 17) and replacement by an 18-nucleotide sequence (bp 81–98) similar in primary sequence to a CanSINE in the opposite orientation. Yellow-highlighted regions mark target site duplications and gray-shaded regions denote the A and B RNA polymerase III recognition sequences. The other puma lineage species, *Acinonyx jubatus* (N = 8) and *P. yagouaround* (N = 8) included the canonical CanSINE profile.

Benchmarking: Which Alignment is Correct?

Thompson JD, Linard B, Lecompte O, Poch O (2011) A Comprehensive Benchmark Study of Multiple Sequence Alignment Methods: Current Challenges and Future Perspectives. PLOS ONE 6(3): e18093. https://doi.org/10.1371/journal.pone.0018093
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0018093

PLOS ONE | TENTH ANNIVERSARY

Statistical Alignment

- Important for full genome comparisons in whole genome sequencing projects where verification by eye not feasible.
- Created to deal with ambiguities within alignment.
- Jointly estimates alignment and phylogeny without potential bias of guide tree used in progressive alignments.
- All possible alignments are considered.
- Bayesian framework to assess confidence using posterior probabilities.
- Recent 2017 study by Bugusz & Whelan (Syst. Biol) indicates performances differ over divergence time.

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A Sampling of Statistical Alignment Programs

BAli-Phy: Bayesian Alignment and Phylogeny Estimation

BEAST: Bayesian Evolutionary Analysis Sampling Trees

Alifritz: Simultaneous Statistical Multiple Alignment and Phylogeny Reconstruction

DART: DNA, Amino and RNA Tests

StatAlign

PRANK: Probabilistic Alignment Kit

PaHMM-Tree

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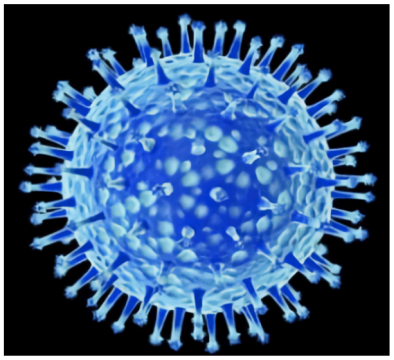
Tutorial Exercise: The Nectin4 Gene in Mammals

Morbilliviruses and host receptors: A case study with Nectin4 (poliovirus receptor-related protein 4 PVRL4) and Canine Distemper Virus

Canine Distemper Virus



Deadly Outbreaks In Lions

A lion with myoclonus, involuntary muscle spasms, possibly associated with previous infection with canine distemper virus during a 1994 outbreak.
Image Credit: Serengeti Carnivore Disease Project

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Our Question: Is Host receptor Nectin4 correlated with neurologic form of CDV infection in felids and canids?

Background:

There are seven known Morbilliviruses that infect Carnivores, Cetaceans, Phocids, Primates, ungulates, small ruminants:

- Feline morbilliviruses
- Canine distemper virus
- Phocine morbillivirus
- Cetacean morbillivirus
- Rinderpest virus
- Small ruminant morbillivirus
- Measles morbillivirus

CDV is known to access cell entry into the host through host genes SLAM and NECTIN4 (PVRL4). It is hypothesized that NECTIN4 is linked with neurological forms of canine distemper. We will conduct a comparative genomic analysis of NECTIN4 in mammals to determine structure, function and evolution. Our study will focus only on the coding regions (CDS) of the gene.

Pilot Study Using Genome Mining:

We have searched RefSeq and NCBI for CDS (exons) of Nectin4 from affected mammalian taxa and downloaded representative sequences that are full-length CDS of NECTIN4.

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Tutorial is on Desktop Folder: Alignment_Exercises

Open Folder

Delete “Alignment_Exercises_Instructions” in folder

Follow Correct “Alignment_Exercises_Instructions” on Github link

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